Reference: *Biol. Bull.* **172:** 83–88. (February, 1987)

LOSS AND RECOVERY OF LOCOMOTOR BEHAVIOR AFTER CNS LESIONS IN THE SNAIL *MELAMPUS BIDENTATUS*

KEITH SNYDER AND STACIA MOFFETT

Department of Zoology, Washington State University, Pullman, Washington 99164-4220

ABSTRACT

To analyze neural pathways underlying the control of locomotion in the snail *Melampus bidentatus,* we lesioned connectives and commissures involved in locomotor control. The results suggest the presence of an independent oscillator in each pedal ganglion which is connected primarily via the pedal commissure. The bilaterally paired cerebropedal and cerebropleural connectives carry redundant information concerned with initiation of locomotion. In the 2–4 weeks after lesions were made, the snails usually regained locomotor coordination.

INTRODUCTION

Gastropod molluscs are favorable models for investigations of neural control of locomotion because of the relative simplicity of their nervous systems. Several features common to locomotion of many opisthobranch and pulmonate gastropods have been discovered using CNS lesions. These include: (1) command cells in cerebral ganglia that initiate and maintain the locomotor behavior, and (2) pattern generators that organize rhythmic output to muscles. Command centers have been found in cerebral ganglia and pattern generators in the pedal ganglia (Fredman and Jahan-Parwar, 1980, 1983; Jahan-Parwar and Fredman, 1980; Lennard *et al.,* 1980; von der Porten *et al.,* 1982; Getting, 1983; Arshavsky *et al.,* 1985a; Satterlie, 1985).

The use of CNS lesions also revealed the gastropod potential for neural repair and associated recovery of specific behaviors (Price, 1977; Arshavsky *et al.,* 1985b; Moffett and Snyder, 1985). The pulmonate gastropod *Melampus* locomotes by means of a "crawl-step" consisting of several discrete components which make it particularly suitable for behavioral analysis after CNS lesions (Moffett, 1979). The alteration of this behavior following CNS lesions has allowed us to explore the role of particular parts of the nervous system in generation and coordination of locomotion. The sequence and timecourse of recovery from the lesions were also recorded.

MATERIALS AND METHODS

Animals and surgical techniques

Snails (*Melampus bidentatus*) with shell lengths of 6–10 mm were obtained from Wollaston, Massachusetts, and Poquoson, Virginia. Animal maintenance, anesthesia, and surgical techniques were as described by Moffett and Snyder (1985).

Lesions

The primitively unfused nervous system of *Melampus* contains five paired ganglia and one unpaired visceral ganglion. Five types of lesions were performed: (1) transec-

Received 25 June 1986; accepted 28 October 1986.

tion of the pedal commissure, (2) transection of both cerebral and pedal commissures, (3) bilateral transection of cerebropedal and cerebropleural connectives and statocyst nerve, (4) isolation of the left pedal ganglion by cutting the left cerebropedal and cerebropleural connectives, the statocyst nerve, and the pedal commissure, and (5) excision of the left pedal ganglion (Fig. 1).

Behavioral analysis

The locomotor behavior of each snail was tested 4 to 7 days after surgery and every 2–4 days thereafter. The snail was placed on a glass plate clamped above an angled mirror which allowed us to view and videotape pedal movements. We determined what portion (%) of the foot surface functioned in locomotion by superimposing a grid on the image of the foot. Even when their crawl was abnormal due to lack of coordination or failure of regions of the foot to participate, snails were able to move along the substratum.

Behavioral deficits fell into two categories: (1) failure of musculature in one region of the foot to participate in locomotion (a musculature control deficit), or (2) a deficit in coordination of locomotor movements in different parts of the foot, such as asymmetrical progression of the pedal wave on the right and left sides of the foot. All observations were made by an experimenter who did not know which surgery the snail had undergone.

Results

Cerebropedal and cerebropleural connectives cut

When cerebropedal and cerebropleural connectives were severed bilaterally (Fig. 1B), snails withdrew into their shells upon recovery from anesthesia. About half did not emerge until they could crawl normally. Although these animals were the slowest of all experimental groups to initiate locomotion after surgery (Fig. 2), they exhibited the fastest recovery of normal pedal waves (Fig. 3). Most deficits in metapodial symmetry in this group involved failure to activate musculature in the posterior third of the foot. They also exhibited abnormal coordination of the propodial region (Fig. 1B).

Pedal commissure cut

All 11 snails which had their pedal commissures cut were crawling by day 7 (Fig. 2). All showed lack of control over the posterior portion of the metapodium on one side (5 animals) or both sides (6 animals), but involvement of the entire foot in locomotion was gradually re-established (Fig. 3). After the entire metapodial musculature became involved in locomotion, the crawl-step was still initiated asymmetrically by contraction of the foot tip on the right or left (Fig. 1C). Once the tip shortened on one side, the pedal wave continued forward either symmetrically or with the initiating side leading. Of 11 snails, 4 regained normal locomotion within 32 days.

Pedal and cerebral commissure cut

When the cerebral commissure was severed in addition to the pedal commissure, side-to-side coordination of pedal locomotion was completely disrupted (Fig. 1D). This surgery delayed the initiation of spontaneous crawling behavior longer than pedal commissurotomy alone (Fig. 2). The time required to re-establish control over

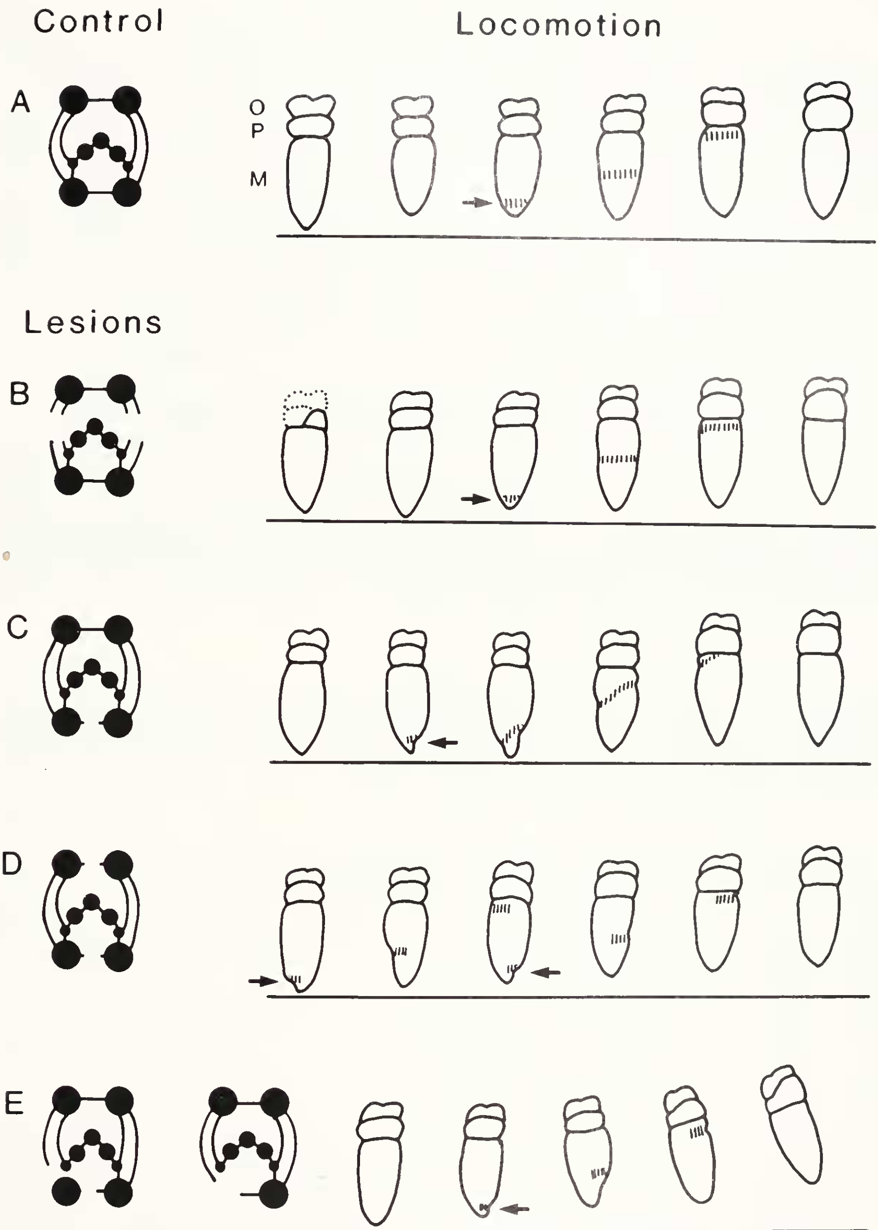

FIGURE 1. Locomotor deficits resulting from CNS lesions. Each line depicts a complete cycle of the crawl-step. Regions on the foot marked by parallel lines are contracting. (A) Control CNS and corresponding locomotion. (O = oral veil, P = propodium, M = metapodium). (B) Right and left cerebropedal and cerebropleural connectives cut. Propodium is often elevated but metapodial wave is normal when snails first locomote. (C) Pedal commissure cut. Wave initiated asymmetrically. (D) Cerebral and pedal commissures cut. Waves on right and left sides independent. (E) Left pedal ganglion isolation and ablation. Each wave pulls animal toward ablated side, requiring continuous course correction. Arrows indicate initiation of pedal wave.

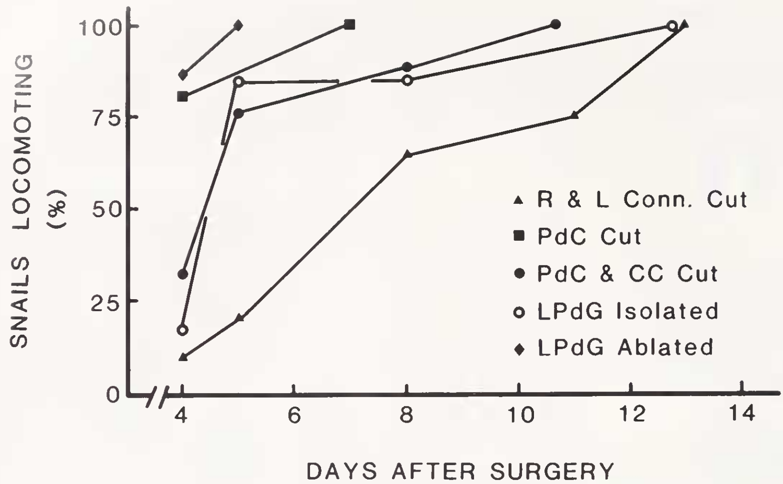

FIGURE 2. Onset of initial attempts at locomotion in groups of snails recovering from each of the five experimental lesions. Triangle = right and left cerebropleural and cerebropedal connectives cut. Square = Pedal commissure cut. Circle (filled) = pedal and cerebral commissures cut. Circle (open) = left pedal ganglion isolated. Diamond = left pedal ganglion ablated.

pedal musculature was similar for snails with pedal commissurotomies and those with pedal plus cerebral commissurotomies (Fig. 3). Animals with both commissures transected initially showed less propodial expansion. Recovery began with the anterior portion of the foot and progressed posteriorly.

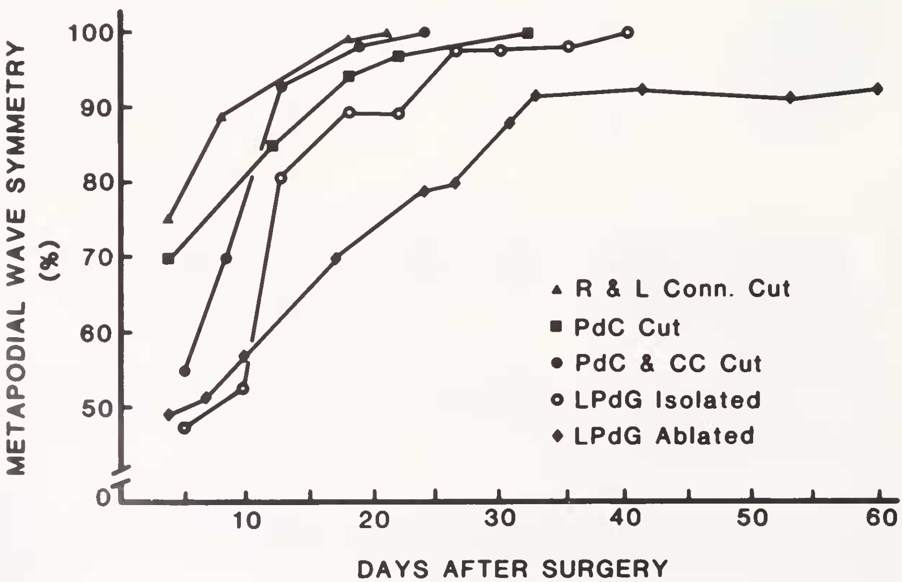

FIGURE 3. Recovery of metapodial wave symmetry in the five experimental groups. Symbols and abbreviations given in Figure 2.

Left pedal ganglion isolated

The ganglion was held in place by its pedal nerves after its central connections were cut. The right side of the foot functioned normally, but the left half of the metapodium failed to participate in locomotion. One animal also exhibited abnormal coordination of a small portion of the right posterior metapodium. The six snails that received this lesion were slower to begin crawling than snails with previously described operations (Fig. 2). The left propodium and the anterior-most portion of the metapodium were the areas in which recovery was first seen. Control over pedal musculature was regained in an anterior to posterior progression, and complete pedal waves were seen on the operated left side in all 6 animals by day 40.

Left pedal ganglion ablated

The most severe operation performed in terms of CNS damage was excision of an entire pedal ganglion (Fig. 1E). Behavior was similar to that following ganglion isolation. All 25 snails were crawling by day 5, although pedal musculature on the left side was inactive and the right posterior metapodial tip was also inactive in one-fourth of the snails. Recovery resembled that following left pedal ganglion isolation, except that it progressed more slowly and only 5 of 25 snails exhibited complete recovery (Fig. 3). The remaining snails all had some region on the left side of the foot that failed to be activated when tested on postoperative day 60.

Discussion

Neurons that carry controlling information from cerebral ganglia to pedal ganglia have been classified as "command" neurons in *Aplysia* (Jahan-Parwar and Fredman, 1978), *Pleurobranchaea* (Gillette *et al.,* 1978), and *Lymnaea* (McCrohan, 1984). When both cerebropleural connectives were cut in *A. californica,* normal crawling continued and only the escape locomotion was abolished (Jahan-Parwar and Fredman, 1979). If both cerebropedal connectives were cut, only limited movements were generated; locomotion stopped entirely when all four connectives were severed. Transection of cerebropedal connectives only slows the locomotor rate in *Clione* (Satterlie and Spencer, 1985). In *Melampus,* signals travelling through either the cerebropleural or cerebropedal connectives are sufficient to initiate locomotion (Snyder, 1986). In the work reported here, snails with bilateral connective transections did not emerge from their shells and crawl as early as the other groups. The timescale for their onset of locomotion could have allowed for connective regeneration (Snyder, 1986).

In addition to interactions between cerebral and pedal levels, side-to-side coordination is important for locomotion. When the pedal commissure was cut in *Clione* or *Aplysia,* the right and left sides of the animal moved asynchronously (Jahan-Parwar and Fredman, 1980; Arshavsky *et al.,* 1985a; Satterlie and Spencer, 1985). In *Melampus,* cutting the cerebral commissure alone had no detectable effect on locomotion (Moffett and Snyder, 1985), whereas cutting the pedal commissure alone was almost as devastating as cutting both commissures.

Ablation of a pedal ganglion results in the same central lesions as pedal ganglion isolation, but also includes loss of a large population of neurons. The degree of recovery we observed following this lesion was therefore quite remarkable, especially considering that the recovery occurred before new neurons are likely to have been generated (Snyder, 1986). For all operations, the rate of behavioral recovery was rapid, considering distance between ganglia and the fact that we had cut neural trunks rather than crushing them, so growing axons had no mechanical linkage to follow in finding their targets. Only animals which had the left pedal ganglion removed failed to regain complete control over pedal musculature.

ACKNOWLEDGMENTS

We wish to acknowledge NSF's MARC Program support of Beverly D. Livingston, who assisted us in data collection, support of S. M. by R01 NS22896 and K. S. by Sigma Xi Grant-in-Aid of Research and a Grant-in-Aid for Graduate Research from WSU.

LITERATURE CITED

Arshavsky, Y. I, I. N. Beloozerova, G. N. Orlovsky, Y. V. Panchin, and G. A. Pavlova. 1985a. Control of locomotion in marine mollusc *Clione limacina* II. Rhythmic neurons of pedal ganglia. *Exp. Brain Res.* **58:** 263–272.

Arshavsky, Y. I, I. N. Beloozerova, G. N. Orlovsky, Y. V. Panchin, and G. A. Pavlova. 1985b. Control of locomotion in marine mollusc *Clione limacina* I. Effective activity during actual and fictitious swimming. *Exp. Brain Res.* **58:** 255–262.

Fredman, S. M., and B. Jahan-Parwar. 1980. Role of pedal ganglia motor neurons in pedal wave generation in Aplysia. *Brain Res. Bull.* **5:** 179–193.

Fredman, S. M., and B. Jahan-Parwar. 1983. Command neurons for locomotion in *Aplysia. J. Neurophys.* **49:** 1092–1117.

Getting, P. A. 1983. Mechanisms of pattern generation underlying swimming in *Tritonia.* II. Network reconstruction. *J. Neurophys.* **49:** 1017–1035.

Gillette, R., M. P. Kovac, and W. J. Davis. 1978. Command neurons in *Pleurobranchaea* receive synaptic feedback from the motor network they excite. *Science* **199:** 798–801.

Jahan-Parwar, B., and S. M. Fredman. 1978. Control of pedal and parapodial movements in *Aplysia.* II. Cerebral ganglion neurons. *J. Neurophys.* **41:** 609–620.

Jahan-Parwar, B., and S. M. Fredman. 1979. Role of interganglionic synaptic connections in the control of pedal and parapodial movements in *Aplysia. Brain Res. Bull.* **4:** 407–420.

Jahan-Parwar, B., and S. M. Fredman. 1980. Motor program for pedal waves during *Aplysia* locomotion is generated in the pedal ganglia. *Brain Res. Bull.* **5:** 169–177.

Lennard, P. R., P. A. Getting, and R. I. Hume. 1980. Central pattern generator mediates swimming in *Tritonia.* II. Initiation, maintenance, and termination. *J. Neurophys.* **44:** 165–173.

McCrohan, C. R. 1984. Initiation of feeding motor output by an identified interneurone in the snail *Lymnaea stagnalis. J. Exp. Biol.* **113:** 351–366.

Moffett, S. 1979. Locomotion in the primitive pulmonate snail *Melampus bidentatus:* foot structure and function. *Biol. Bull.* **157:** 306–319.

Moffett, S., and K. Snyder. 1985. Behavioral recovery associated with the central nervous system regeneration in the snail *Melampus. J. Neurobiol.* **16:** 193–209.

von der Porten, K., D. W. Parsons, B. S. Rothman, and H. Pinsker. 1982. Swimming in *Aplysia brasiliana:* analysis of behavior and neuronal pathways. *Behav. Neural Biol.* **36:** 1–23.

Price, C. H. 1977. Regeneration in the central nervous system of a pulmonate mollusc, *Melampus. Cell Tiss. Res.* **180:** 529–536.

Satterlie, R. A. 1985. Reciprocal inhibition and postinhibitory rebound produce reverberation in a locomotor pattern generator. *Science* **229:** 402–404.

Satterlie, R. A., and A. N. Spencer. 1985. Swimming in the pteropod mollusc, *Clione limacina.* II. Physiology. *J. Exp. Biol.* **116:** 205–222.

Snyder, K. A. 1986. Regeneration and locomotor behavior after CNS lesions in *Melampus bidentatus.* Ph.D. Dissertation, Washington State University, Pullman.